The Role of Charge in Protein Adsorption at Surfaces

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In the present study, insulin and the two chains that make up the insulin molecule (chain A and B) were used as a model system for further developmental investigations of the interfacial behaviour of the larger proteins. Studies were made to compare the two electrochemical techniques of cyclic voltammetry and electrochemical impedance spectroscopy (EIS) measurements on the adsorption behaviour of these molecules at the platinum surface. The electrode/ electrolyte interface and corresponding surface processes were modeled by applying an equivalent-electrical-circuit (EEC) approach.

A phosphate buffer solution, 0.05 M pH 7.0, was used as an electrolyte. Solutions of bovine insulin were prepared by dissolving the reagents in the phosphate buffer. Conductivity water (Nanopure, resistivity of 18.2 MΩ cm) was used in the preparation of all aqueous solutions. All measurements were carried out in well-stirred oxygen-free solutions. A standard three-electrode cell was utilized: the working and counter electrodes were made of platinum of high purity (99.99%, Johnson-Matthey) and the reference electrode was a saturated calomel electrode (SCE). A temperature range of 273 to 353 K (0 - 80 ^{o}C) was used in the experiments.

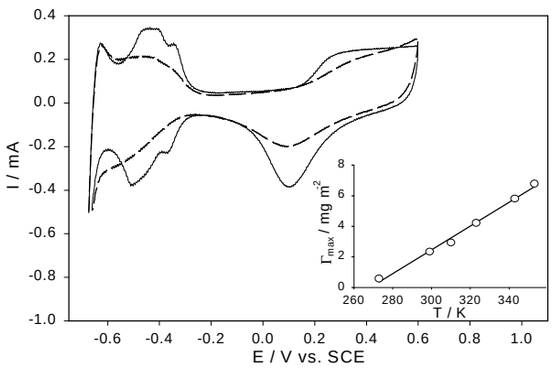

Fig. 1. Cyclic voltammetry of a Pt electrode in phosphate buffer, pH 7.0, recorded (———) in a protein-free solution, and (- - - - -) with addition of insulin (0.17 g L^{-1}). Scan rate: $\nu = 500$ mV s^{-1}, temperature: $T = 310$ K. *Inset:* Dependence of the maximum surface concentration on temperature.

Cyclic voltammetry measurements (Fig.1) have shown that the surface charge density resulting from protein adsorption is directly proportional to the amount of adsorbed proteins (surface concentration),[1-3] indicating that adsorption at anodic potentials is accompanied by the transfer of charge, that is, chemisorption through carboxylate groups on the protein. Fig. 1 shows that with the increase in concentration of insulin in the bulk solution, the surface concentration sharply increases and reaches a plateau value. Also, with increase in temperature, the plateau values linearly increase (inset in Fig. 1). The surface concentration of chain A was greater than that for insulin, due to the difference in the size of the molecule (smaller A chains are able to pack closer together than the whole insulin molecule). The surface charge density of chain B was closer to that of insulin, probably due to the dimerization of chain B in the solution. The experimentally determined surface concentration of insulin molecules at 310 K was 2.9 mg m^{-2}, which is very close to the theoretical value of 2.7 mg m^{-2}. The larger experimental value may be due to the partial unfolding of insulin on the surface.

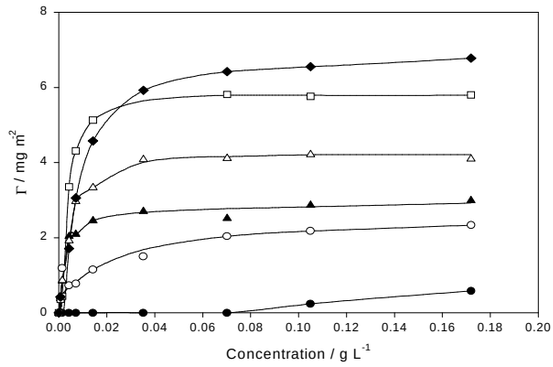

Fig. 2. Surface concentration of insulin on platinum in phosphate buffer pH 7.0 at: (●) 273 K, (○) 299 K, (▲) 310 K, (Δ) 323 K, (□) 343 K and (◆) 353 K.

Electrochemical impedance spectroscopy data have been modeled using the Randles equivalent electrical circuit. The polarization resistance was found to be sensitive to the surface concentration of the protein and it decreased with increase in the concentration of protein in the bulk solution. This indicates that the adsorption of protein is accompanied by the transfer of charge.

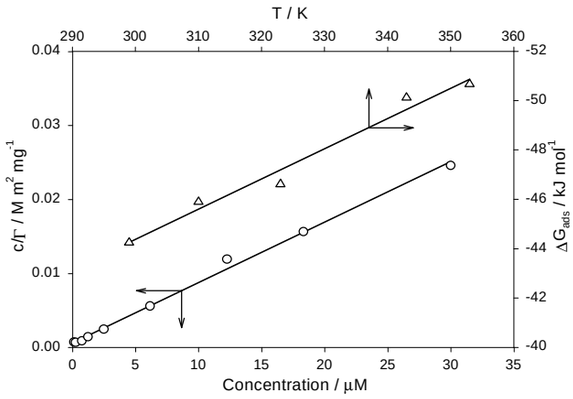

Fig. 3. (Δ) Adsorption isotherm of insulin adsorbed onto a Pt surface in phosphate buffer pH 7.0 at 310 K and (○) dependence of the Gibbs free energy of adsorption on the temperature.

The adsorption processes have been described using the Langmuir adsorption isotherm (Fig.3). High negative values for the Gibbs free energy were obtained with each of the molecules at all temperatures (Fig.3), indicating strong adsorption onto the platinum surface. The positive entropy of adsorption for insulin (130 J mol^{-1} K^{-1}) indicates that insulin partially unfolds on the surface. The relatively small negative value of enthalpy (-4.6 kJ mol^{-1}) indicates that the net influence of enthalpy on adsorption of insulin on Pt is minor under the experimental conditions applied in our experiments. Hence, the adsorption process was found to be entirely governed by the change in entropy. All the thermodynamic values determined from cyclic voltammetry data and electrochemical impedance spectroscopy were found to agree within experimental uncertainty.

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